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THE PRESENCE OF SEROTONIN IN SOME
SPECIES OF GENUS *URTICA*

IVAN REGULA and ZVONIMIR DEVIDE

(Department of Botany, Faculty of Science, University of Zagreb)

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Introduction

Indole amines which are derived from tryptophan occur in a variety of plant families but are especially common in *Leguminosae* (Banerjee and Ghosal 1969, Bowden et al. 1954, Fellows and Bell 1971, Ghosal et al. 1971, 1972).

The investigation of indolealkylamines in the family *Urticaceae* has indicated that some species, such as *Urtica dioica* (Collier and Cheshier 1956), and *U. pilulifera* var. *dodartii* (Regula 1974), contain serotonin (5-hydroxytryptamine), while *U. pilulifera* in addition to serotonin contains its methylated derivative — bufotenin (Regula 1970, 1972).

In this paper the presence of serotonin in some other species of the genus *Urtica* namely *U. membranacea*, *U. thunbergiana*, *U. cubensis*, *U. ferox* and *U. urens* is investigated.

Experimental

The stems with leaves and the seeds of species *U. membranacea*, *U. thunbergiana*, *U. cubensis*, *U. ferox* and *U. urens* were separately homogenized with cold methanol (+ 3°C) in a blender. Plant material was extracted four times with the same solvent at low temperature. The extracts were concentrated under mild conditions (+ 33°C and 18 mm Hg). Plant pigments and lipophilic substances in the extracts were removed with petroleum ether. The extracts were then passed through a column of cation exchanger — Amberlite CG-50 in NH_4^+ form. The columns were washed with 0.02 N ammonium acetate and the basic substances eluted with 1 N hydrochloric acid.

Ammonium acetate effluents were concentrated to dryness and the residues dissolved in methanol and applied on columns of Dowex-50 X4

Table 1. Rf values and colour reactions of the compound from extract and sample of 5-hydroxytryptamine

Substance	Paper chromatography Rf in solvent system*						Thin layer chromatography	Reagents						
	1	2	3	4	5	6 ⁺	6 ⁺⁺	7 ⁺	I	II	III	IV	V	VI
Substance	0.48	0.52	0.36	0.62	0.09	0.66	0.80	0.13	b.	b.	b.	v.	v.	v.
5-Hydroxy-tryptamine	0.48	0.52	0.36	0.63	0.08	0.66	0.80	0.13	b.	b.	b.	v.	v.	v.
*1. n-BuOH-AcOH-H ₂ O	(60 : 15 : 25)						I = Ehrlich's							
2. i-PrOH-NH ₃ -H ₂ O	(10 : 1 : 1)						II = p-Dimethylaminocinnamaldehyde							
3. n-BuOH-EtOH-H ₂ O	(4 : 1 : 1)						III = Xanthidrol							
4. MeOH-BuOH-C ₆ H ₆ -H ₂ O	(4 : 2 : 2 : 2)						IV = 1-Nitroso-2-Naphthol							
5. Dest. H ₂ O							V = Ninhydrin							
6. i-PrOH-NH ₃ -EtAc	(35 : 20 : 45)						VI = Ninhydrin-Acetic Acid							
7. CHCl ₃ -C ₆ H ₆	(1 : 1)						b. = blue							
+ SiO ₂ G							v. = violet							
+ + Al ₂ O ₃ G														

in H^+ form. These columns were eluted with water and then with 2 N NH_4OH . Indolic substances were detected and identified by paper and thin layer chromatography as well as by spectrophotometry and spectrophotofluorimetry.

Results and Discussion

In the extracts of *Urtica membranacea*, *U. thunbergiana*, *U. cubensis*, and *U. ferox* we noticed two indolic substances. One of them was eluted from Amberlite CG-50 with hydrochloric acid. The R_f values of this substance on paper and thin layer chromatography as well as colour reactions with 1-nitroso-2-naphthol, ninhydrin-acetic acid and other reagents (table 1) were identical with those of the authentic sample of serotonin. The identity of this substance was confirmed also by measurements of UV spectra in neutral solution (max. 275 nm and 295 nm) and fluorescence in strong acid (activation at 295 nm and fluorescence at 550 nm). The amounts of serotonin in the stems with leaves and the seeds are shown in Table 2.

Table 2. Amounts of serotonin in the *Urtica* species

Species	Stems with leaves ($\mu g\ g^{-1}$ fr. w.)	Seeds
<i>U. membranacea</i>	0.26	+
<i>U. thunbergiana</i>	0.31	+
<i>U. cubensis</i>	0.42	+
<i>U. ferox</i>	0.33	+
<i>U. urens</i>	0.00	?

As can be seen, all species except *U. urens* contain serotonin in the vegetative organs investigated and in the seeds. The amounts of seeds available, however, were too small for quantitative measurements.

In the ammonia effluents from the Dowex column of the leaf and stem extracts we also detected tryptophan. Histochemical reaction with 6% p-Dimethylamino-benzaldehyde in conc. hydrochloric acid on the nettle sting and the cross-section of seeds gave a positive reaction (except *U. urens*), i. e. a characteristic blue-green colour given also by the authentic sample of serotonin.

Conclusion

In extracts of the stems with leaves and of the seeds of *Urtica membranacea*, *U. thunbergiana*, *U. cubensis* and *U. ferox* we proved the presence of serotonin by chromatography, spectrophotometry and spectrophotofluorimetry. The positive histochemical reaction inside the nettle sting and the seeds demonstrated that serotonin was localized also in these parts of plants. In *U. urens* this indolyethylamine was not found either in the vegetative organs investigated or in the seeds.

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SAŽETAK

PRISUTNOST SEROTONINA U NEKIM VRSTAMA RODA *URTICA**

Ivan Regula i Zvonimir Devidé

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Stabljike s listovima te posebno sjemenke pojedinih vrsta su homogenizirane i ekstrahirane hladnim metanolom. Ekstrakti su izmučkavani petrol-eterom i propuštanj preko kolona ionskih izmjenjivača Amberlita CG-50 i Dowexa-50 X4. Serotonin je identificiran kromatografijama na papiru i tankim slojevima, spektrofotometrijski i spektrofotofluorimetrijski. Histokemijskom reakcijom je utvrđeno da se serotonin nalazi u dlakama žeravkama i sjemenkama. Na temelju tih istraživanja je utvrđeno da: *Urtica membranacea* sadržava u stabljici i listovima 0.26 ($\mu\text{g g}^{-1}$ svježe tvari) serotonin, *U. thunbergiana* 0.31, *U. cubensis* 0.42 i *U. ferox* 0.33. Kod vrste *U. urens* nije ni u jednom slučaju mogla biti utvrđena prisutnost serotoninina.

Dr. Ivan Regula
Prof. dr. Zvonimir Devidé
Department of Botany
Faculty of Science
University of Zagreb,
Rooseveltov trg 6/III, P. O. Box 933
YU-41001 Zagreb (Jugoslavija)

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